Sterilization efficacy of Mars UV and return cruise environment on Martian dust particles

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Objectives of this section

Objective 1: To investigate the sterilization potential of Mars UV on dust that could be collected on the exterior surfaces of an Orbiting Sample (OS)

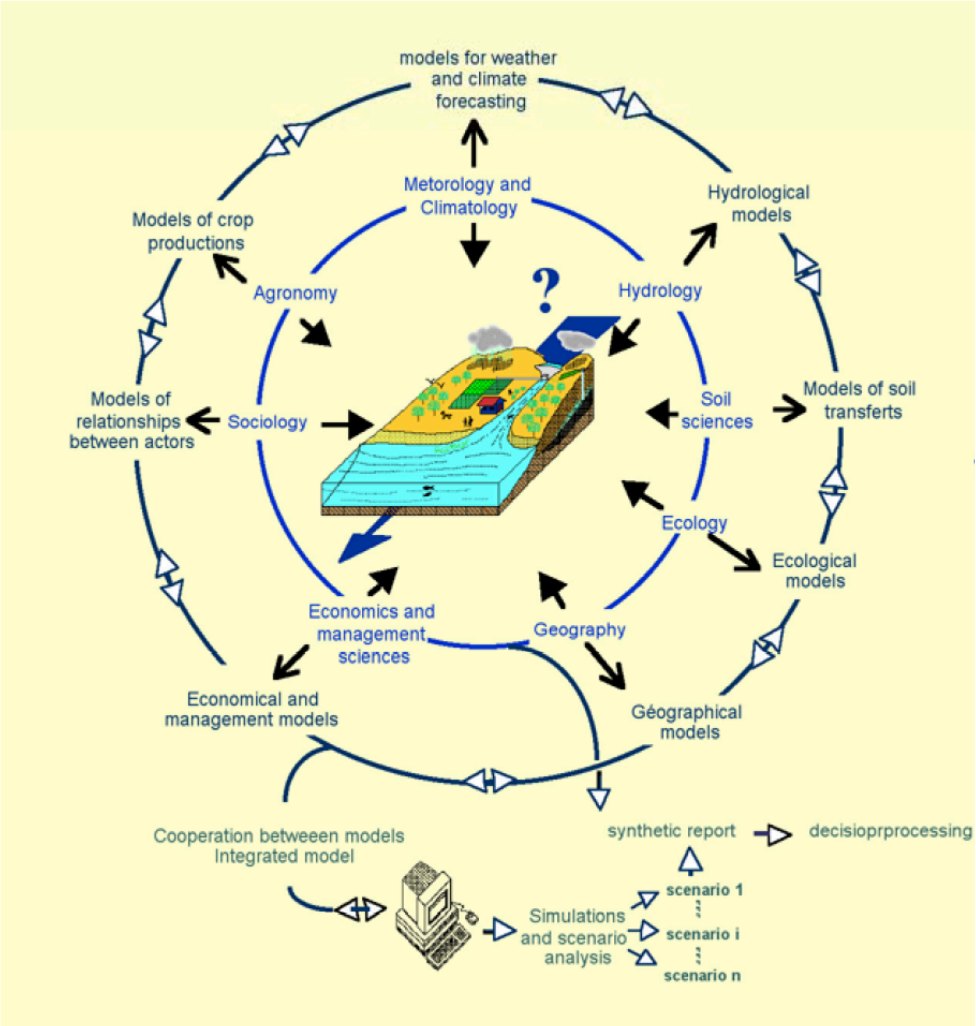
Objective 2: To investigate the sterilization potential of the Mars-to-Earth cruise environment on dust that could be collected on the exterior surfaces of an Orbiting Sample (OS)

Objective 1:
To investigate the sterilization potential of Mars UV on dust that could be collected on the exterior surfaces of an Orbiting Sample (OS)

The fundamental challenge
Complexity of natural systems

“The complexity of natural systems means that they are more than the simple sum of their constituent parts. They display a non-linear dynamical behaviour and an hierarchical organisation, and are also capable of self-organisation. Adopting integrative scientific approaches to analysis is a prerequisite to understanding this complexity and finding appropriate solutions.”

-Barbault, R. et al. 2004. Integrative biology and complexity of natural systems: keys to addressing emerging challenges.



A conceptual example of an integrated modelling approach to address a question regarding environmental/biological interactions. (Barbault, R., et al., 2004)

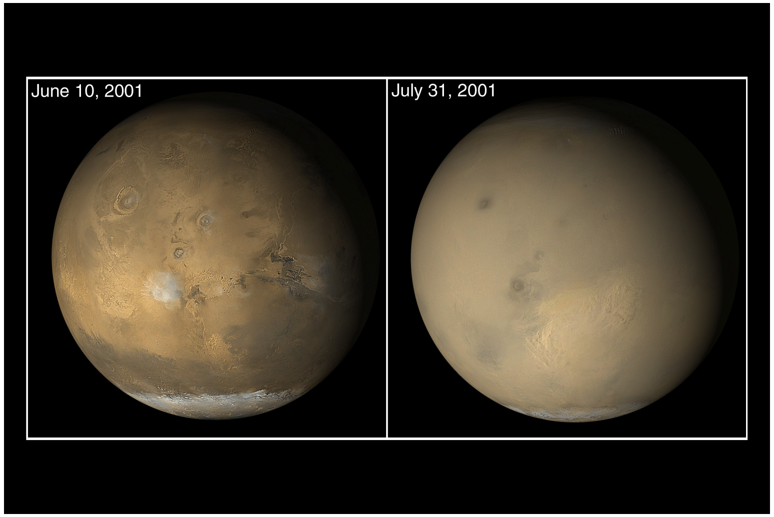
Mars 2020 UV Model Review
Summary

- Review held July 12, 2017
- Presenters: Ira Katz, David Crisp
- Review Board Members:
  - Peter Smith, Chair , Professor Emeritus, University of Arizona
  - Mark Lemmon, Associate Professor, Texas A&M University
  - Michael Mischna, JPL
  - Carlos Soares, JPL
- Mars 2020 UV Model Goal: Calculate on Mars 2020 rover surfaces the UV dose that can inactivate Viable Organisms (VOs) brought from Earth
- Review goal: Assess the applicability and adequacy of the Mars 2020 UV code models:
  - Mars surface solar irradiance
  - Rover geometry
  - Direct and indirect radiation
- From the Board Report:

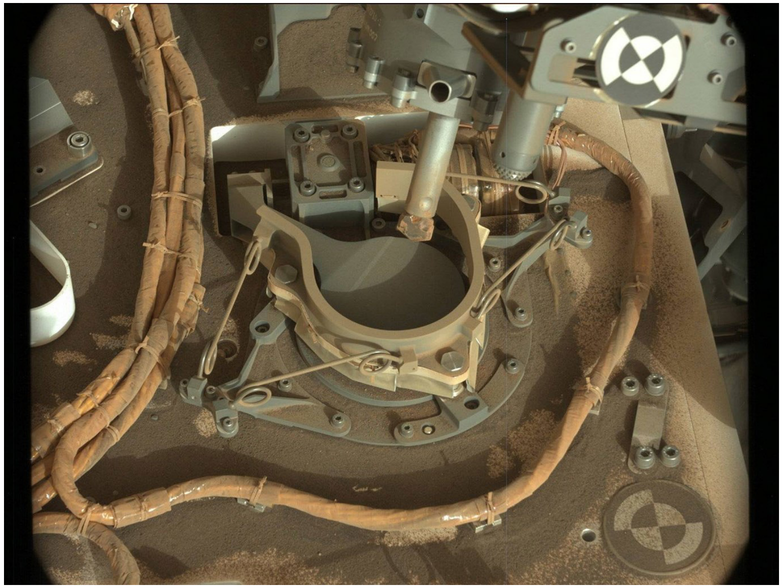
  "The UV presentations showed that years (more than 20) of development of the atmospheric model, used for many planetary atmospheres, have resulted in an excellent tool for understanding the UV fluence striking all the Mars 2020 rover exposed surfaces. The combination of the UV radiation with the susceptibility of various microbes to the radiation results in an ability to calculate the kill rate. The model has the flexibility to account for rover and instrument shape irregularities and estimate the time to reach the 99% value in directly or indirectly illuminated regions."
- Note from the Board Chair: "frankly your modeling effort is very advanced and has few weaknesses"

Mars UV environment

- Sunlight will irradiate both aerosolized and deposited Martian dust particles
- Exposure to this solar radiation can be through direct, diffuse or reflected ultraviolet (UV) radiation
- Particle exposure to UVC will vary by a variety of factors.



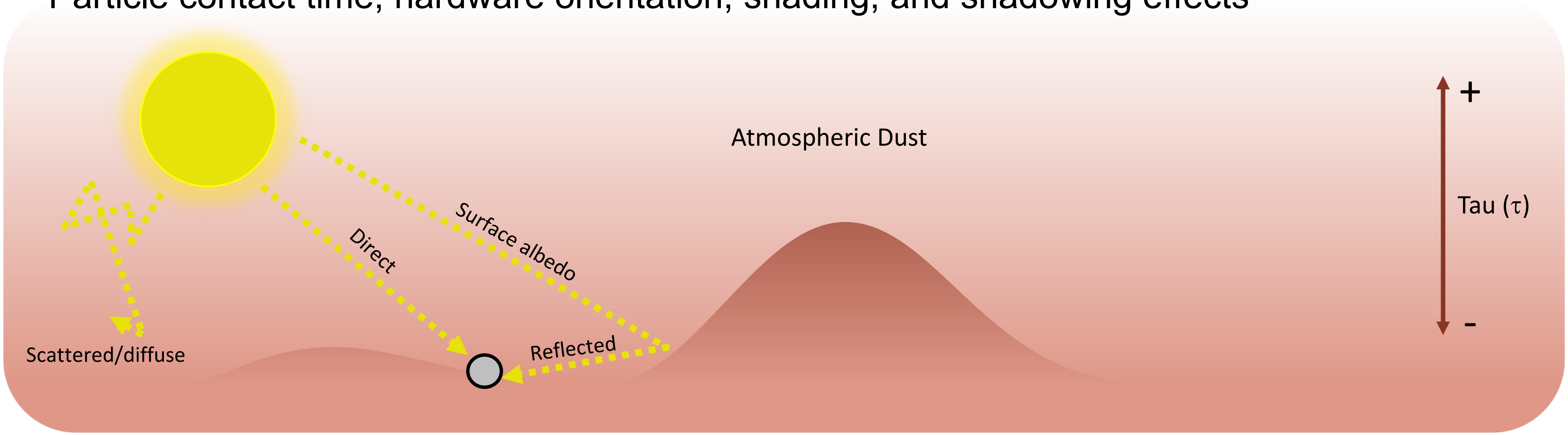
Mars under clear conditions vs. Mars during a dust storm.
Courtesy: NASA



Dust deposition on the MSL rover
Courtesy: NASA/JPL/Texas A&M/Cornell

Factors influencing UV exposure and dose rate

- Structure and composition of the Martian atmosphere
  - Humidity, tau, gas mixing ratios (Katz, I. Mars UV Code Review, 2018)
- Optical properties of Mars dust and surface particles
  - Produces effects on the single scattering albedo of Mars dust aerosols and surfaces
    - The direct solar flux dominates
      - at low solar zenith angles (i.e. nearing noon)
      - for low dust optical depths (measured in tau [τ])
    - The diffuse solar flux dominates when
      - the sun is near the horizon
      - the dust optical depths are large
- Spatial (geographic location), and temporal (day of the year, local time, etc.) heterogeneities.
- Particle contact time, hardware orientation, shading, and shadowing effects



Investigating the sterilization potential of Mars UV

In 2017, an exhaustive review of > 100 peer reviewed studies spanning 50+ years of research on UV inactivation of microbial species was conducted for Mars 2020 to:

1. Determine the scientific consensus of Mars UV sterilization
2. Understand the limitations of UV as a sterilization modality
3. Compile microbial UV lethality data to serve as inputs into the Mars 2020 UV model

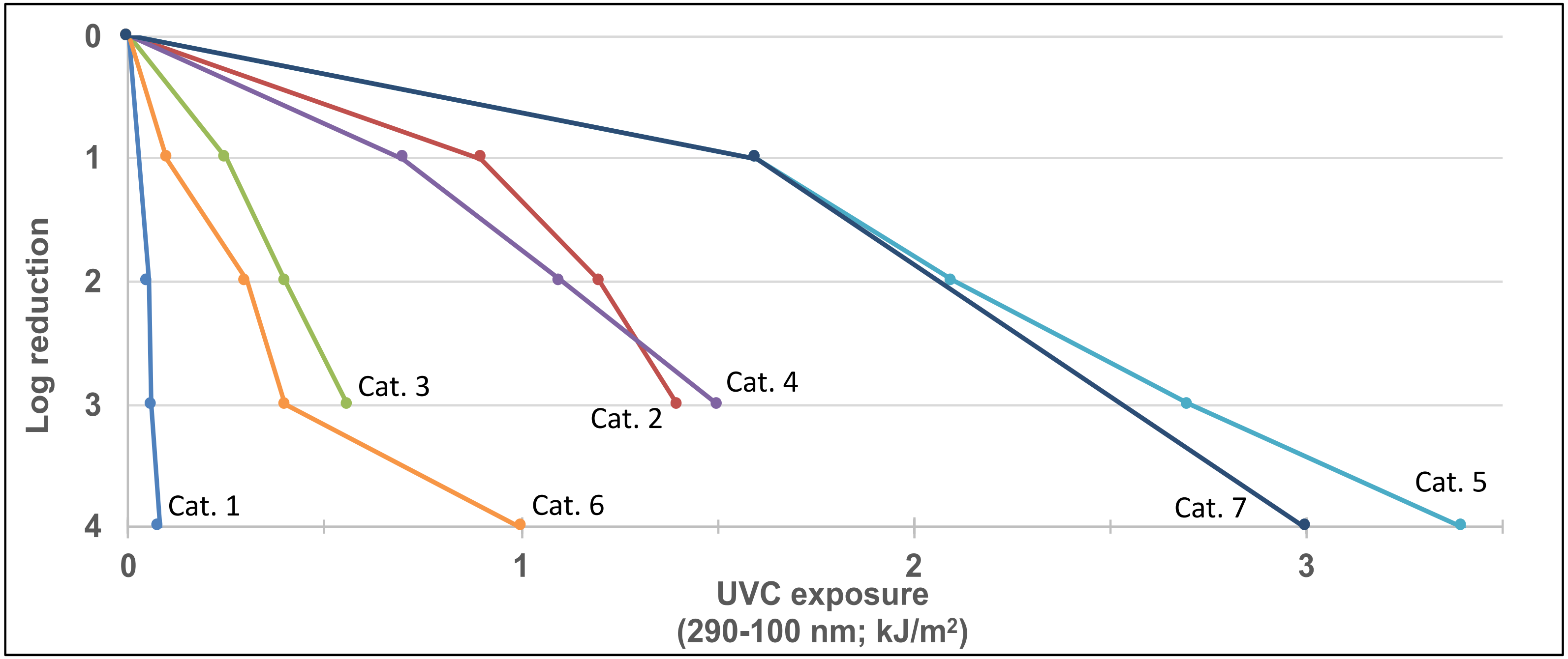
Q: How do we accurately assess the UV potential on Mars?

A: Use the Mars 2020 UV and cruise mortality model example.

1. Examine available data to understand microbial responses to UV
2. Examine available data to understand the UV environment on Mars

Assumption regarding UV sterilization
The effects of UV exposure on potential Mars microorganisms is comparable to that of Earth microorganisms.

Microbial response to UV exposure
UV lethality curves



Category	Organism Type
1	Culturable, vegetative, non-radiation resistant bacteria[1]
2	Highly radiation resistant non-spore forming vegetative bacteria[4,6]
3	Typical bacterial spores[2]
4	Moderately UV resistant bacterial spores[2]
5	Highly UV resistant spores[5]
6	Typical fungal conidia[3]
7	Radiation resistant fungal conidia[3]

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2. Link, L., et al. 2004. Extreme spore UV resistance of Bacillus pumilus isolates obtained from an ultraclean spacecraft assembly facility. *Microbial ecology* 47.2: 159-163.
3. Blachowicz A, Knox BP, Romsdah IJ, Palmer JM, Huttenlocher A, Wang CCC, Keller NP, Venkateswaran K: Characterization of *Aspergillus fumigatus* isolated from air and surfaces of the International Space Station. In: *13th European Conference on Fungal Genetics: April 3-6, 2016; Paris, France, April 3-6, 2016.* 2016.
4. Slade and Radman, 2011. Oxidative stress resistance in Deinococcus radiodurans. *Microbiol Molec Biol Rev* 75(1): 133-191.
5. Horneck, G., et al. 2012. Resistance of bacterial endospores to outer space for planetary protection purposes—experiment PROTECT of the EXPOSE-E mission. *Astrobiology* 12.5: 445-456.
6. Vaishampayan P, Roberts AH, Augustus A, Pukall R, Schumann P, Schwendner P, Mayilraj S, Salmassi T, Venkateswaran K: *Deinococcus phoenicis* sp. nov., an extreme ionizing-radiation-resistant bacterium isolated from the Phoenix Lander assembly facility. *Int J Syst Evol Microbiol* 2014, 64(Pt 10):3441-3446.

Mars UV environment
MSL REMS, Pathfinder, and M2020 Mars UV model data

Mars 2020 UV Model vs. MSL REMS Data
Green line – Mars UV code
Red & blue lines – REMS data

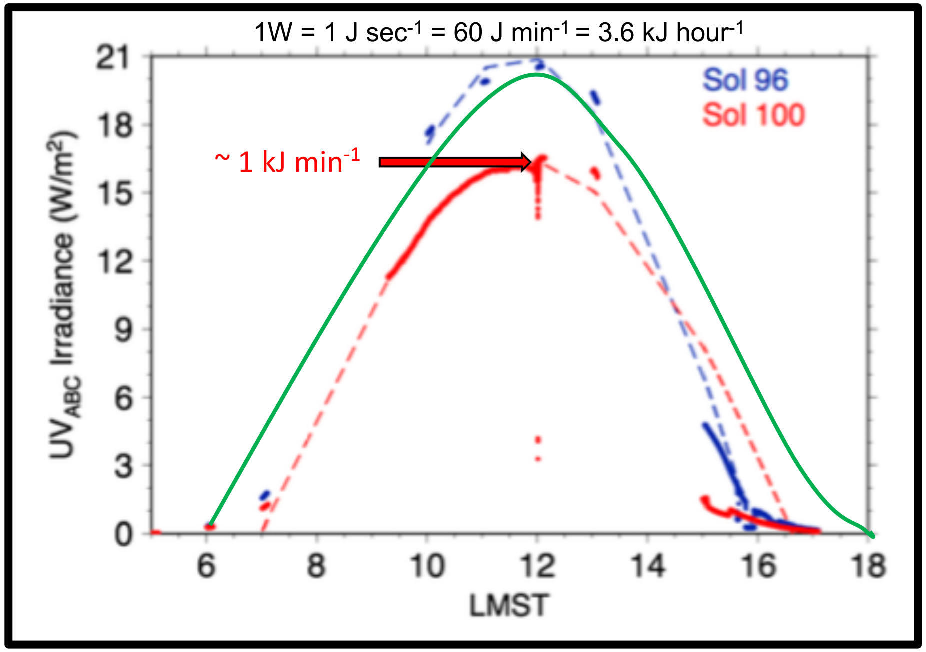


Figure 3. Diurnal cycles of UVABC irradiance on sols 96 and 100. Tau = 2.0, E_UV sol^{-1} = 433 kJ/m (Gomez-Elvira et al., 2014)

In ~ 16 min. after sunrise, an exposed microbe would have already received over 4 kJ of UV.

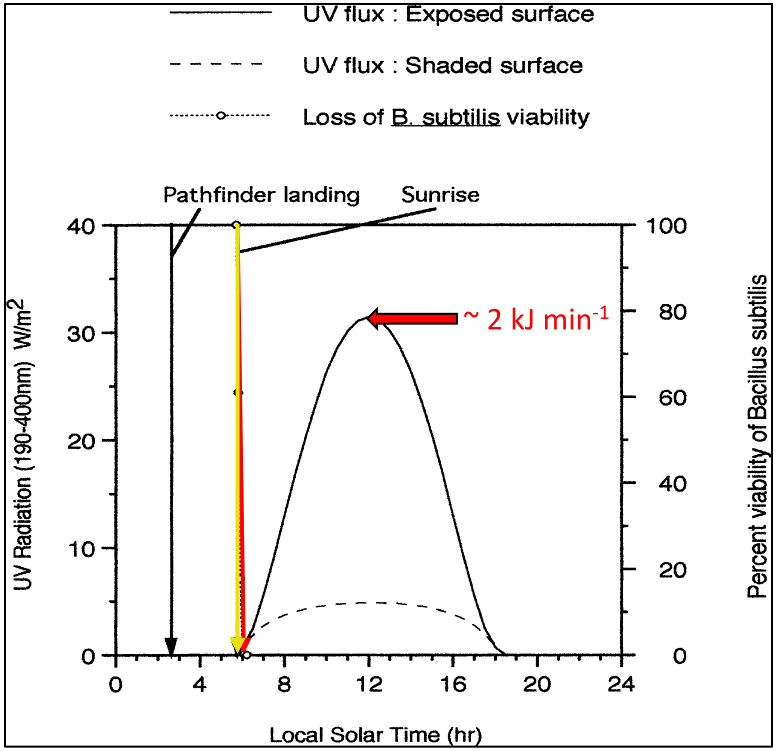


Figure 5. UV flux on Pathfander Sol 1. (Cockell, 2000)

Martian UV will quickly inactivate 99.99% of exposed spores, but not all surfaces of the rover will receive full UV exposure

Surface UV sterilization
UV shielding from dust deposition on surfaces

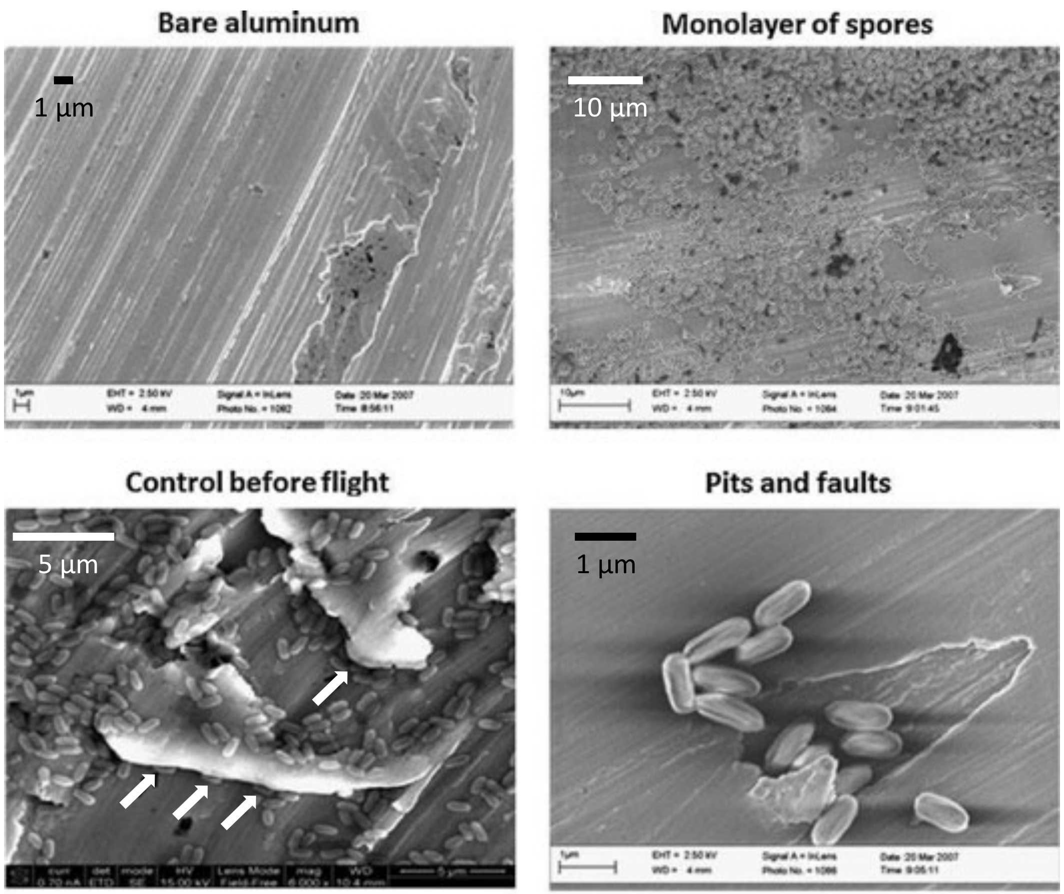
- The available data indicates that even the most resistant UV microorganism we have discovered, would be reduced by 99.999% from Mars UV minutes after its initial exposure.
- A number of studies have determined that "UV radiation was the key parameter that determined survivability of spores under simulated Martian conditions; direct exposure to UV radiation resulted in rapid and nearly complete inactivation of microbial cultures." (Nicholson et al., 2005).
- Furthermore...
  - "... thin (tens of micrometers) to thick (centimeters) contiguous layers of Mars analog soils were generally adequate for protecting a significant proportion of test populations of spores from the lethal effects of UV irradiation... , individual particles of Mars analog dust measuring up to 50m in diameter deposited over bacterial monolayers (a condition that more accurately simulates aeolian dust settling onto a spacecraft surface than thick contiguous layers) were found not to protect endospores of B. subtilis from the UV flux of a simulated Martian spectrum."
- Caveat:
  - Where UV does not shine...the microbes [may] do just fine.

Surface UV sterilization
UV shielding from dust deposition on surfaces

- Mars dust deposition can provide spores with a respite from UV exposure
  - Mancinelli, Rocco L., and Melisa Klovstad. "Martian soil and UV radiation: microbial viability assessment on spacecraft surfaces." *Planetary and Space Science* 48.11 (2000): 1093-1097.
    - 1mm covered – survival near 100%
    - 0.5 mm covered – 60% survival
    - 12 µm covered – 10% survival
  - Newcombe, David A., et al. "Survival of spacecraft-associated microorganisms under simulated martian UV irradiation." *Applied and environmental microbiology* 71.12 (2005): 8147-8156.
    - "...airborne dust loading is an important component that attenuates incoming solar UV in the Martian atmosphere."
    - "Optical depths on Mars of 0.3 (clear-sky) to 3.5 (global dust storm) would correspondingly produce approximately 25–97% attenuations..."
  - Schuerger, Andrew C., et al. "Rapid inactivation of seven Bacillus spp. under simulated Mars UV irradiation." *Icarus*181.1 (2006): 52-62.
    - "... rapid inactivation of terrestrial bioloads on spacecraft components may be possible on Sun-exposed surfaces on Mars, and that this process may occur faster than the aeolian dust can accumulate on these surfaces."

Surface UV sterilization
UV shielding from dust deposition on surfaces

- Electron micrographs of *Bacillus pumilus* SAFR-032 spores on aluminum coupons.
- Arrows in "control before flight" panel indicate presence of spores under uneven surfaces of the coupon which provide protection from UV radiation.
- EXPOSE-E PROTECT Experiment



Vaishampayan, et al., 2012

Surface UV sterilization
UV shielding from dust deposition on surfaces

- Sphere = Analogue for rocks and particles on the rover deck
- E_UVC exposure (kJ/m^2) under a Mars dust particle for 1 Sol
- A particle deposited on the surface can provide shielding from UV to microbes on the surface.

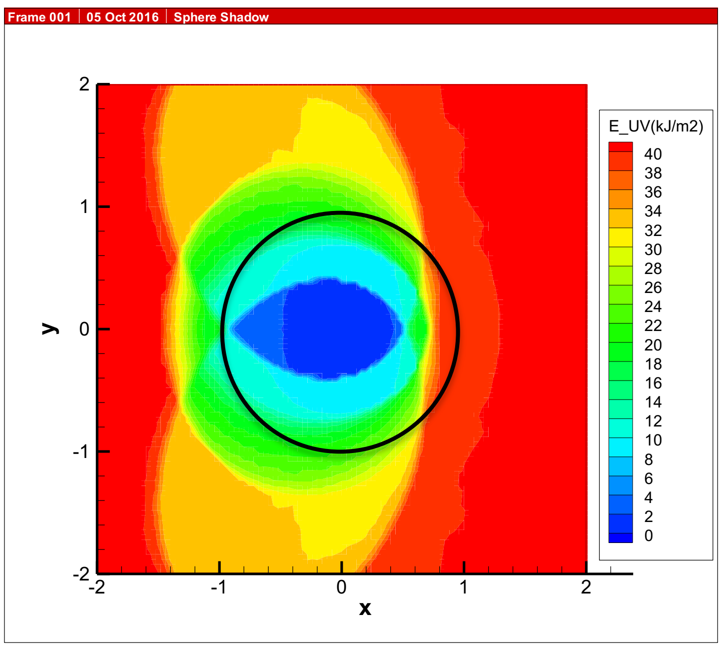
Fraction of sphere projected area :

3.1% sees less than 25 J/m^2

4.2% sees less than 500 J/m^2

7.2% sees less than 1 kJ/m^2

26% sees less than 4 kJ/m^2



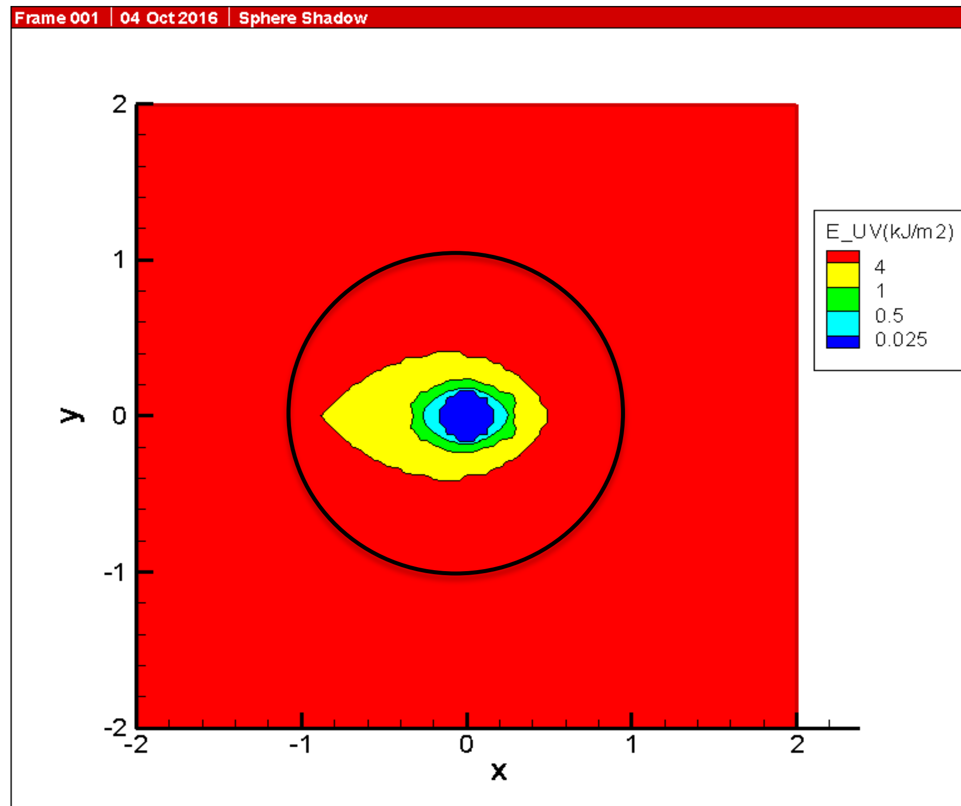
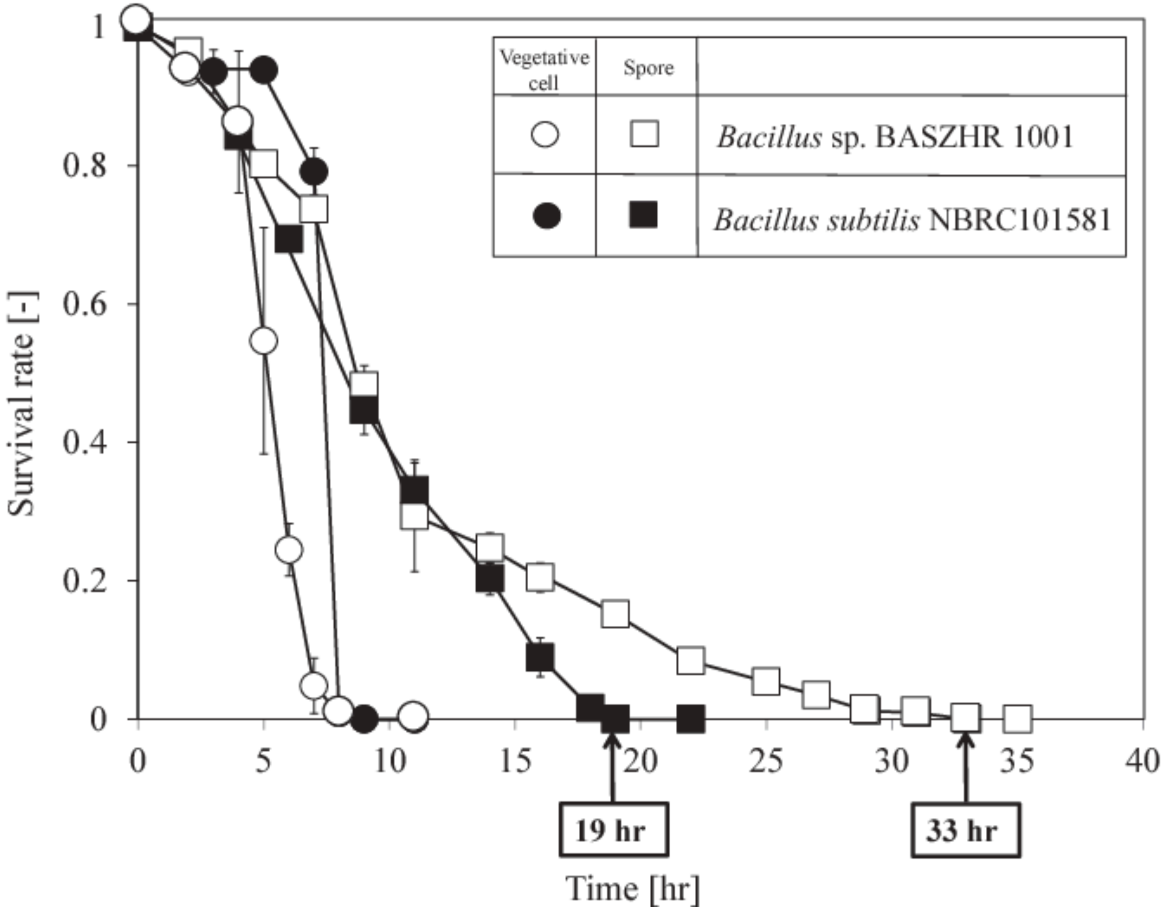


Image: Ira Katz, Mars 2020 UV Model Review, July 12, 2017

Aerosol UV sterilization
Microbial survivability in dust associated aerosols

- Kellog and Griffin, 2006
  - Dust-associated bacteria can be transported over 5000 km from Africa to the Caribbean.
  - It is a misconception that all microorganisms in dust clouds are killed by solar UV-radiation, lack of nutrients and desiccation during their multi-day journey.
  - "Four studies of African dust identified...Most of the bacteria are Gram positive, and many are spore-formers, making them more resistant to desiccation."
  - Much of the bacteria isolated from aerosol samples are highly pigmented, which indicates microbial adaptations to resist UV radiation exposure.
- Kobayashi et al., 2014
  - The results of this UV irradiation experiment showed that UV radiation tolerance of both Kosa bioaerosol bacterial vegetative cells and bacterial spores was higher than that of their soil vegetative cell and spore counterparts.

Aerosol UV sterilization
Microbial survivability in dust associated aerosols



Kobayashi, Fumihisa, et al. "Bioprocess of Kosa bioaerosols: Effect of ultraviolet radiation on airborne bacteria within Kosa (Asian dust)." *Journal of bioscience and bioengineering* 119.5 (2015): 570-579.

- The effects of UV radiation on the survival rate of Kosa bioaerosol, *Bacillus* sp. BASZHR 1001 and soil bacterium, *Bacillus subtilis* NBRC 101581.
- The UV radiance was approximately 20.1 0.1 W/m^2.
- Symbols indicate vegetative cells (open circles), spores (open squares) of Kosa bioaerosol, Bacillus sp. BASZHR 1001, vegetative cells (closed circles) and spores (closed squares) of soil bacterium, Bacillus subtilis NBRC 101581.

Effect of shadowing on non-spore formers and spore survival

- Osman et al. (2008)
  - Simulated the shadowing effect of soil particulates on bacterial survival under simulated Martian atmospheric and UV irradiation conditions.
  - Almost all cells prepared in 60 µm size particles survived.
  - Spores associated with 0.2 µm size particles associated survived exposure to full Martian UV irradiation displayed full lethal effects within ~5 min.
- Schuerger et al. (2003)
  - Demonstrated that individual dust particles measuring up to 50 µm in diameter had only a minor protective effect on the survival of *B. subtilis* spores when exposed to UV irradiation under simulated Mars conditions.

Dust on surface of the MSL rover
Courtesy: NASA/JPL

Summary
UV sterilization efficacy on Mars

UV sterilization efficacy on spacecraft surfaces

- Current scientific consensus suggests that UV conditions on Mars can be highly lethal to spores deposited on surfaces.
  - A conservative 4-log reduction (99.99%) of the heartiest spores currently known can be obtained within 15 minutes of exposure.
- The degree of shielding and shading appears to be the primary limiting factor for UV sterilization on Mars.
  - Spores can escape the lethal effects of UV by "hiding" in pits, under overhangs, under other spores, beneath mm-scale layers of dust.
  - UV irradiation on exposed spacecraft surfaces appears to be a race against time in which UV lethal dosages must be accumulated faster than the daily dust load can cover and protect the adhered or embedded microbes.

UV sterilization efficacy in dust aerosols

- Terrestrial studies indicate bacterial spores and cells associated with dust aerosols can exhibit mechanisms which increased their resistance to the damaging effects of UV radiation.
- We need to gain a much better understanding on the effects of UV on spore aerosols.

Probability models can help generate a final confidence level to determine the efficacy of UV sterilization on both aerosolized and surface deposited particles in Mars conditions.

Objective 2:
To investigate the sterilization potential of the Mars-to-Earth cruise environment on dust that could be collected on the exterior surfaces of an Orbiting Sample (OS)

Mars cruise microbial mortality
Juno vs. Mars 2020

"...a complete model on how spore-forming and non-spore forming terrestrial microorganisms might survive transport to other planetary bodies has not yet been fully developed. Thus, the potential for the movement of microorganisms among the various planetary bodies within our Solar System remains poorly constrained." (Nicholson et al., 2005).

- The interplanetary (cruise) environment between Earth and Mars is composed of several factors that impact the survival of microorganisms[1-3]
  - High vacuum
  - Severe desiccating conditions
  - Extreme temperature variations
  - Solar UV irradiation
  - Solar particle events
  - Galactic cosmic rays (GCR)
- For Mars 2020, a background study was performed to investigate spore survivability during cruise.
- The "Juno Model" for cruise survivability was revisited and revised for Mars 2020

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2. Nicholson et al. 2005.The solar UV environment and bacterial spore UV resistance: considerations for Earth-to-Mars transport by natural processes and human spaceflight. Mutation res. 571: 249-264.
3. Horneck, G., et al. 2012. Resistance of bacterial endospores to outer space for planetary protection purposes—experiment PROTECT of the EXPOSE-E mission. *Astrobiology* 12.5: 445-456.

Mars cruise microbial mortality

Juno and Mars 2020

Category	Organism Description	Juno cruise survival %[1]	M2020 cruise survival %
1	Culturable, vegetative, non-radiation resistant bacteria	10	10[1]
2	Highly radiation resistant non-spore forming vegetative bacteria	50	50[1]
3	Typical bacterial spores	100	30[2]
4	Moderately UV resistant bacterial spores	100	100[3]
5	Highly UV resistant spores	100	100[3]
6	Typical fungal conidia	NA	100[4]
7	Radiation resistant fungal conidia	NA	100[4]

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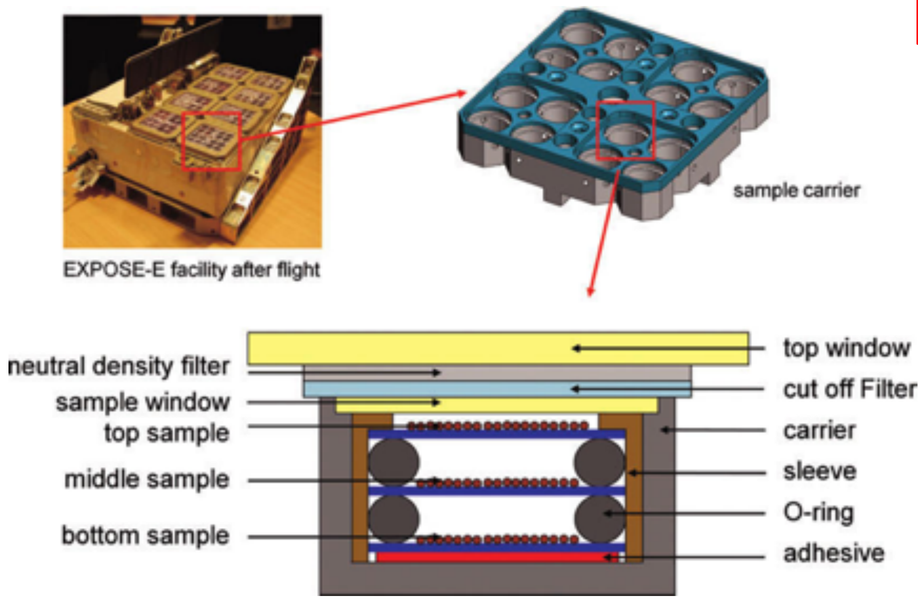
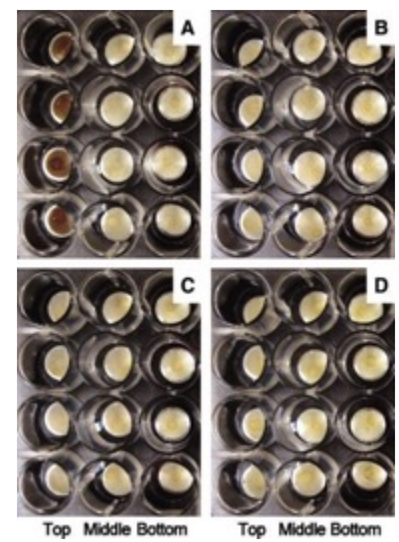
2. Nicholson et al. 2005.The solar UV environment and bacterial spore UV resistance: considerations for Earth-to-Mars transport by natural processes and human spaceflight. Mutation res. 571: 249-264.
3. Vaishampayan P, Roberts AH, Augustus A, Pukall R, Schumann P, Schwendner P, Mayilraj S, Salmassi T, Venkateswaran K: *Deinococcus phoenicis* sp. nov., an extreme ionizing-radiation-resistant bacterium isolated from the Phoenix Lander assembly facility. *Int J Syst Evol Microbiol* 2014, 64(Pt 10):3441-3446.
4. Blachowicz A,, et al. Characterization of *Aspergillus fumigatus* isolated from air and surfaces of the International Space Station. In: *13th European Conference on Fungal Genetics: April 3-6, 2016; Paris, France.*

EXPOSE-E PROTECT experiment

- EXPOSE-E mission examined *in situ* bacterial endospore survivability on a simulated extended mission to Mars
- *Bacillus* spp. spores were transferred onto spacecraft-qualified aluminum coupons
- Mounted on the balcony of ISS for 1.5 years under multiple UV exposure treatments

"…in situ exposure to outer space is necessary to provide empirical data on microbial survival mechanisms in extraterrestrial environments." (Vaishampayan, et al., 2012)





Horneck, Gerda, et al. "Resistance of bacterial endospores to outer space for planetary protection purposes—experiment PROTECT of the EXPOSE-E mission." *Astrobiology* 12.5 (2012): 445-456.

EXPOSE-E PROTECT
Results-Space conditions

- Dark (completely shielded from UV)
  - *B. subtilis*
    - ~ 0.3-log reduction (55% survival)
  - *B. pumilus* SAFR-032
    - ~ 0.8 log reduction (16% survival)
- Irradiated 100% (fully exposed)
  - *B. subtilis* **(multilayers)**
    - ~ 3-log reduction (< 0.1% survival)
  - *B. pumilus* SAFR-032 **(monolayers)**
    - ~ 6-log reduction (0.13% survival)
- Irradiated 0.1% (shaded)
  - *B. subtilis*
    - ~ 2-log reduction (1.1% survival)
  - *B. pumilus* SAFR-032
    - ~ 3-log reduction (0.13% survival)

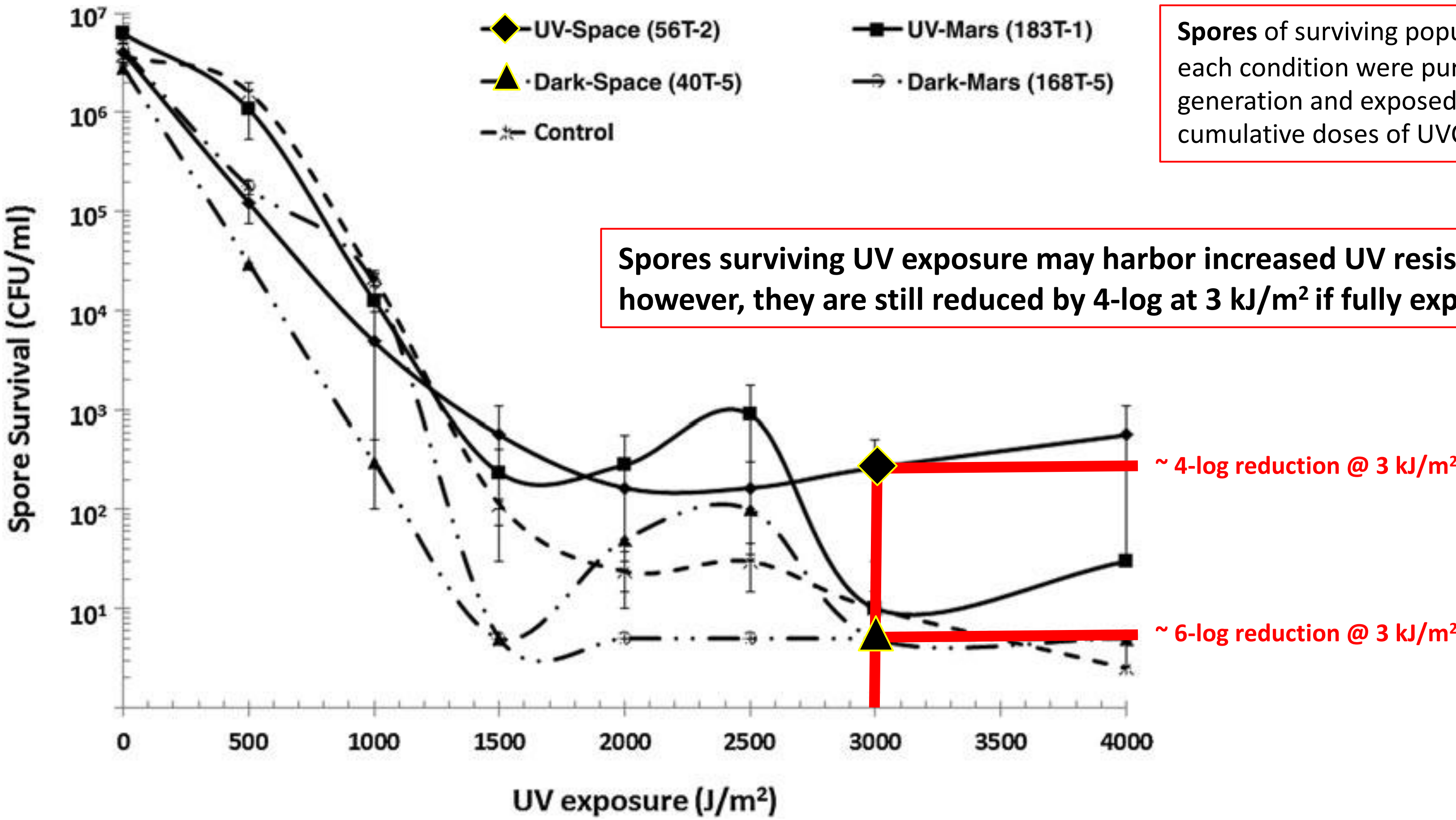
Completely shielded **< 1-log reduction**
Bioburden reduction resulted from exposure to space conditions other than UV (i.e. cosmic radiation, low temperatures, etc.)

Fully exposed **3 – 6-log reduction**
These results indicate that shielding can have greater effect of spore survival than species-specific resistance

Shaded **2 - 3-log reduction**

Results summarized from Horneck et al., 2012

EXPOSE-E PROTECT
Results-Space conditions



Spores of surviving populations from each condition were purified from first generation and exposed to various cumulative doses of UVC (254nm).

Spores surviving UV exposure may harbor increased UV resistance; however, they are still reduced by 4-log at 3 kJ/m² if fully exposed after

Horneck, Gerda, et al. "Resistance of bacterial endospores to outer space for planetary protection purposes—experiment PROTECT of the EXPOSE-E mission." *Astrobiology* 12.5 (2012): 445-456.

Microbial survivability after an Earth-Mars cruise and 1 sol of UV exposure on Mars

Category	Organism Description	Abundance	Cruise Survival	Post Cruise	1-log	2-log	E>log 1	E >log 2	UV survival	Survival x abundance
1	Culturable, vegetative, non-radiation resistant bacteria	85.5%	10%	8.55	0.05	0.05	96.3%	96.3%	4.6%	0.395
2	Highly radiation resistant non-spore forming vegetative bacteria	0.01%	50%	0.005	0.9	1.2	70.0%	65.4%	31.1%	0.002
3	Typical bacterial spores	10%	30%	3	0.25	0.4	92.0%	87.7%	9.3%	0.279
4	Moderately UV resistant bacterial spores	1.5%	100%	1.5	0.7	1.1	77.6%	66.2%	24.2%	0.363
5	Highly UV resistant spores	1%	100%	1	1.6	2.1	63.7%	60.3%	37.2%	0.372
6*	Typical fungal conidia	1%	100%	1	0.1	0.3	95.5%	90.7%	5.9%	0.059
7*	Radiation resistant fungal conidia	1%	100%	1	1.6	3	63.7%	55.9%	37.6%	0.376
	Initial	100%	**Cruise Survival**	16%					**Cruise & UV Survival**	**1.85%**

(Brian Shirey & Kasthuri Venkateswaran, “Biological inputs to Mars UV model”, September 21, 2017)

Summary

Sterilization efficacy of the Mars-Earth cruise environment

- Developing an increased understanding of Mars cruise spore survivability required an expansion of the industry-standard single biological indicator scheme
- Seven biological indicator categories were established to account for the range of microorganisms that could be on a spacecraft during cruise
- Microorganisms are capable of surviving a Mars-Earth cruise, and survivability is greatly increased in the absence of exposure to UV [or other sterilizing effect].
- Microorganisms which may survive Mars-Cruise can exhibit higher sterilization resistance mechanisms than laboratory strains.
- We need to gain a much better understanding on the effects of the Mars-Earth cruise environment on spacecraft associated microorganisms

Additional References

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